Original Paper

Biology of the Neonate

Biol Neonate 2001;79:21–26

Single Breath Analysis of Endogenous Nitric Oxide in the Newborn

Andreas Artlich[a,b] Baldvin Jónsson[a] Maneck Bhiladvala[a]
Per Arne Lönnqvist[c] Lars E. Gustafsson[b,d]

[a]Neonatal Research Unit, [b]Physiology at Campus, [c]Paediatric Anaesthesiology and [d]Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

Key Words
Autoinhalation · Exhaled gas · Nasal cycle · Nitric oxide

Abstract
Nitric oxide (NO) is found in the exhaled gas of humans immediately after birth. However, variations of endogenous NO concentration during the breathing cycle have not been studied in newborns. We examined 24 newborns without acute respiratory compromise during spontaneous nasal breathing. Gas was sampled from the tip of a thin nasal catheter placed in the hypopharynx. Endogenous NO concentrations measured by chemiluminescence were assigned to the breathing cycle using synchronized CO_2 recording. Exhaled NO could reproducibly be measured at 1.9 ± 0.2 parts per billion (ppb, mean ± SEM). Autoinhaled nasal NO peaks during regular breathing were 12.0 ± 1.7 ppb and reached intermittent maxima of 52.2 ± 5.8 ppb. During regular breathing 6 infants exhibited sudden decreases of nasal NO peaks to periods with <50% amplitude suggesting transient shortage of autoinhaled nasal NO. We conclude that tidal NO analysis can be used to assess upper and lower airway NO production noninvasively during spontaneous breathing in the newborn.

Introduction

Nitric oxide (NO) is endogenously produced in the lung of most vertebrates and humans and can be detected in the exhaled gas [1, 2]. The largest portion of total NO production in the respiratory tract probably occurs in the nose, particularly in the paranasal sinuses [3–7], whereas a minor portion is released into gas exhaled from the lower respiratory tract [for review, see 2]. Nasally derived endogenous NO is autoinhaled and regarded as an aerocrine messenger influencing ventilation perfusion matching and oxygenation [4, 8, 9]. NO exhaled from the lower airways is significantly increased in asthmatic patients and regarded as a promising noninvasive diagnostic option to assess the severity of asthmatic inflammation in the airways [for review, see 2].

Animal studies have revealed an important role of NO in the perinatal transition of the pulmonary circulation to the high flow/low resistance conditions of extrauterine life [for review, see 10]. However, it is currently not clear how this physiological process is reflected in the exhaled gas. Neonatal measurements in humans have been sparse [11–13]. NO of nasal origin can be found in the exhaled gas of humans immediately after birth [11, 12]. The technical characteristics of NO measurement have, however, precluded the analysis of autoinhaled nasal NO concentra-

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
0006–3126/01/0791–0021$17.50/0

Accessible online at:
www.karger.com/journals/bon

Andreas Artlich
Zentrum für Kinderheilkunde, Justus-Liebig-Universität Giessen
Feulgenstrasse 12, D–35392 Giessen (Germany)
Tel. +49 641 99 43463, Fax +49 641 99 43419
E-Mail andreas.artlich@paediat.med.uni-giessen.de

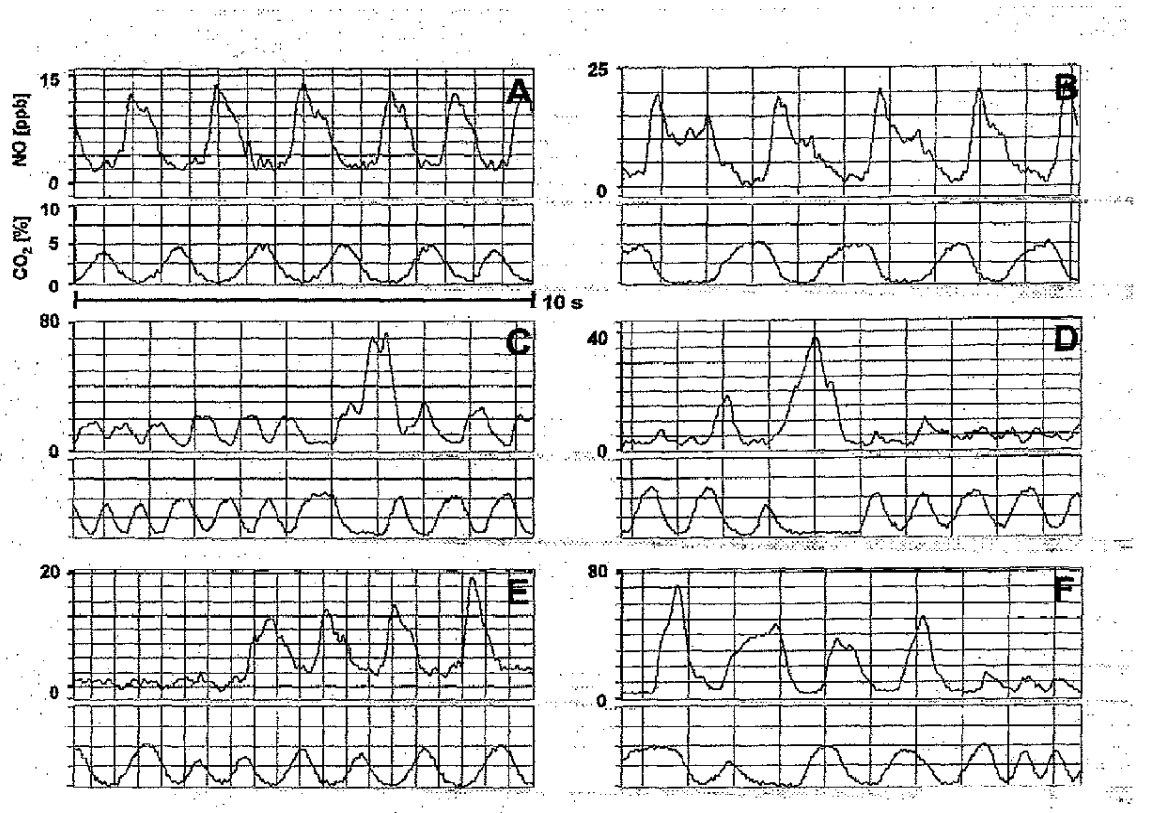

Fig. 1. Synchronized NO and CO_2 measurements in the hypopharynx of newborn infants. **A, B** Regular breathing. **C, D** Intermittent inspiratory NO peaks. **E, F** Sudden changes of inspiratory NO peaks with periods of low inhaled NO concentration. Please note the variable scaling of gridlines on the NO axis.

tions during spontaneous breathing. Moreover, NO exhaled from the lower respiratory tract of infants has only been assessed in mixed exhaled gas [13, 14] or by rapid thoracic compression during pharmacological sedation [15]. We studied autoinhaled nasal and exhaled lower airway NO breath by breath in spontaneously breathing newborn infants.

Patients and Methods

After approval by the local ethical committee and parental consent 24 newborn infants were enrolled in the study. 15 infants had been born prematurely at a median gestational age of 29.1 weeks (range 23.3–34.7), 9 infants had been born at term. 9 infants were recovering from respiratory distress syndrome (RDS), 5 had previously had transient tachypnea of the newborn.

When examined at a median postnatal age of 5.0 days (range 1–25), all infants were under stable respiratory conditions with peripheral oxygen saturations of 88–92% and capillary or venous pCO_2 <7.0 kPa. To achieve this 10 infants were being treated with nasal continuous positive airway pressure (NCPAP) of 2–4 cm H_2O using NO-free pressurized air [<0.5 parts per billion (ppb) NO] and a commercially available system (Infant flow™, Dansjö Medical A/S, Bromma, Sweden) [16]). 7 of these infants had previously required endotracheal intubation and mechanical ventilation for hyaline membrane disease (n = 6) or congenital cystic adenomatoid malformation (n = 1) but had been breathing spontaneously for a minimum of 3 days (mean 8.4 days) after extubation. 6 infants on NCPAP treatment still required additional oxygen with a mean FiO_2 of 0.33

(range 0.24–0.4) being supplied via the CPAP system (n = 5) or by increasing FiO_2 in the incubator (n = 1). All subjects were examined in a supine position and breathed nasally, oral breathing being averted by the use of a pacifier or gentle manual occlusion of the lips. Nasal decongestants had not been applied during at least 24 h prior to the measurements. 12 infants without clinical or hematological signs of systemic infection were being treated with parenteral antibiotics.

To sample gas for analysis, a gastrointestinal feeding tube (outer diameter 0.15 cm) with two holes within 1.5 cm of its tip (Maersk Medical A/S, Lynge, Denmark) was inserted transnasally to a distance of 5–6 cm from the nasal orifice. The tip of the catheter was thus positioned in the pharynx. During CPAP treatment infants were examined using commercially available connectors (Infant flow) which had been fitted with a side port to allow transnasal insertion of the catheter via a gastight lock (hemostasis valve, Arrow, Erding, Germany). Gas was sampled at a flow rate of 190 ml min^{-1} and analyzed for CO_2 and NO concentration using a combined detection system (Aerocrine AB, Danderyd, Sweden). Samples were entered into the analyzers via dehumidifying tubes (Datex Engström AB, Bromma, Sweden). NO measurement by chemiluminescence was calibrated with mass-flow-controlled dilutions (Bronkhorst, Ruurlo, The Netherlands) of a certified NO standard (2 ppm in pure nitrogen, AGA Gas AB, Lidingö, Sweden), the capnometer was calibrated with room air and 5% CO_2 gas (AGA). NO was detected with a rise time of T_{10-90} = 0.2 s and a sensitivity of 1.1 ppb NO, CO_2 was sensed with a rise time of T_{10-90} = 0.3 s and a sensitivity of <0.1% CO_2. NO and CO_2 measurements were synchronized by creating a simultaneous NO and CO_2 signal at the tip of the sampling catheter. For that purpose the tip of the sampling catheter was replaced by an 18-gauge cannula to puncture balloons filled with a suitable gas mixture. Ambient NO concentrations were <5 ppb during measurements.

Measurements were recorded online, traces of 2.5 min being stored for later analysis. Typical recordings are shown in figure 1. Periods of regular breathing as indicated by stable tidal CO_2 measurements for at least 10 breaths were identified. The respiratory rate was then extrapolated from the duration of 10 breaths. As animal experiments indicate that NO measurements might be influenced by preceding alterations of airway CO_2 concentrations [17], only the second half of these periods (fig. 1A, B) was further evaluated: NO concentrations were assigned to the breathing cycle using the synchronized CO_2 signal. Peak NO concentrations during inspiration and trough NO concentrations during expiration were averaged over 5 breaths. Moreover, intermittent inspiratory peak concentrations were documented. Data were evaluated by nonparametric statistical analysis using Mann-Whitney rank sum test and Spearman rank order correlation. Probabilities of $p < 0.05$ were regarded as conveying statistical significance. Results are universally given as mean ± SEM.

Results

During regular breathing (respiratory rate 60 ± 5 and 37 ± 4 breaths min^{-1} in preterm and term infants, respectively) NO peaks during nasal inspiration were 12.0 ± 1.7 ppb. During expiration NO concentrations of 1.9 ± 0.2 ppb were measured.

Expiratory NO levels did not differ significantly between preterm and term infants (p = 0.6), between those infants receiving systemic antibiotic treatment and controls (p = 0.5) or between those infants receiving oxygen supplement or NCPAP and controls (p = 0.8 and p = 0.4, respectively). There was no statistically significant correlation between expiratory NO levels and respiratory rate (p = 0.4), gestational age (p = 0.9), actual body weight (p = 0.6) or postnatal age (p = 0.07).

Inspiratory nasal NO peaks during regular nasal breathing did not differ significantly between those infants receiving systemic antibiotic treatment and controls (p = 0.7). There was no statistically significant correlation between inspiratory nasal NO peaks and respiratory rate (p = 0.2), gestational age (p = 0.5), actual body weight (p = 0.6) or postnatal age (p = 0.9). Intermittently nasal NO peaked at maxima (fig. 1C, D) of 52.2 ± 5.8 ppb which did not correlate significantly with gestational age (p = 0.5), actual body weight (p = 0.3) or postnatal age (p = 0.9). In spite of continued and unchanged tidal CO_2 recording, 6 infants exhibited sudden fluctuations of nasal NO peaks to periods with <50 or >200% amplitude suggesting periodic alterations in the availability of autoinhaled nasal NO (fig. 1E, F, 2). Reversal to the original amplitude of the NO peaks was never seen during the same trace (duration 2.5 min). Mean NO concentrations for the periods of high and low NO concentrations were 14.5 and 2.4 ppb, respectively. Based on this observation, 9 out of the remaining 18 infants could a posteriori be classified as having stable high nasal NO peaks with a mean of 12.2 ppb, 6 showed stable low nasal NO peaks with a mean of 3.3 ppb (fig. 2). The NO concentrations in infants with stable high or stable low concentrations did not differ significantly from the respective concentrations in infants with intermittent high and low concentrations (p = 0.18 and 0.13, respectively). 3 infants were not classified because of continuously unstable nasal NO peaks; 2 infants who had stable high concentrations on 1 day showed stable low concentrations 2 and 3 days later, respectively.

To evaluate the repeatability of NO measurements during regular breathing two measurements taken on the same day and spaced by 2.5–5 min were compared in 16 infants. The mean difference of these NO measurements (2nd to 1st measurement) was 1.2 ± 0.3 ppb (mean ± SEM) for inspiratory NO peaks and 0.2 ± 0.03 ppb for expiratory NO levels, respectively. The corresponding coefficients of repeatability [18] were 3.1 and 0.4 ppb, respectively, indicating good repeatability [18] of NO measurements using tidal analysis.

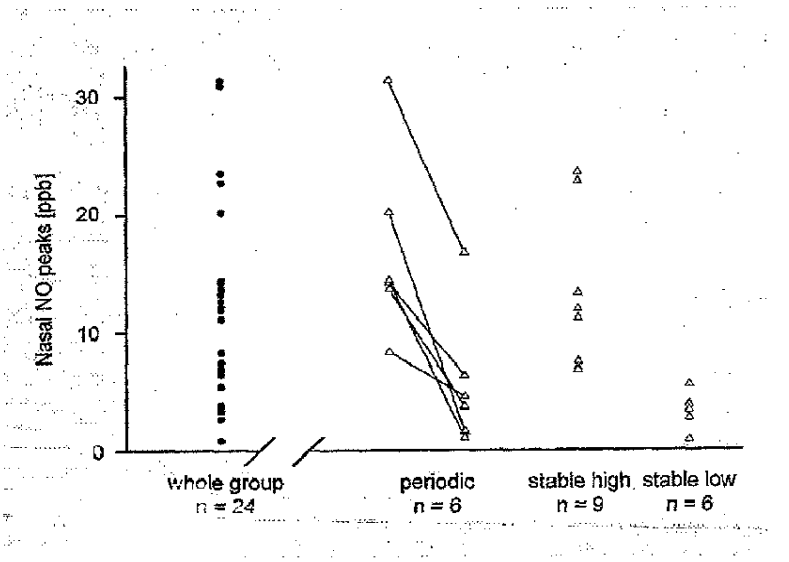

Fig. 2. Inhalational NO peaks during regular breathing in newborns. ● = Original measurements; Δ = a posteriori subdivision of data material trying to explain sudden fluctuations of NO peaks in spite of continued and unchanged tidal CO_2 concentrations in a subgroup of infants (n = 6, see text for details).

Discussion

This is the first account of a tidal analysis of endogenous NO revealing exhaled and autoinhaled concentrations in newborn infants. With the aid of synchronized tidal CO_2 analysis, NO from upper and lower airway sources could be differentiated and reproducibly measured. Exhaled NO concentrations were comparable in all subjects studied whereas autoinhaled NO concentrations were more variable (fig. 2), a subgroup of infants revealing sudden shifts of amplitude (fig. 1E, F, 2).

NO concentrations were measured by sampling gas through a catheter placed in the pharynx. It cannot be entirely excluded that the first exhalation fraction contained residual amounts of nasal or oropharyngeal NO deposited in the dead space during the previous inhalation. However, fractional analysis of exhaled gas in adults has not shown significant differences of NO concentration between the first 40–45% and the remaining part during regular breathing [19], whereas dead space accumulation due to airway NO formation is seen during the arrested airway flow [11, 12, 20]. Measurements during exhalation were thus largely representative of NO exhaled from the lower airways. The values found in neonates in this study (1.9 ± 0.2 ppb) are slightly higher than those found in anesthetized women with healthy lungs (1.3 ± 0.2 ppb) [7]. Apart from age-related phenomena this difference could either be due to a small contribution by NO produced nonenzymatically from saliva [21] or due to the fact that prior lung disease (RDS, transient tachypnea of the newborn) continued to mildly stimulate lower airway synthesis in spite of clinical restitution. The repeatability of exhaled NO measurements in neonates has not previously been assessed. Using the present technique NO measurements were well repeatable and may in the future facilitate the longitudinal study of exhaled NO in pulmonary disorders of the neonate.

Autoinhaled NO concentrations during quiet breathing in neonates have previously been reported by Schedin et al. [11]. In contrast to intermittent peaks of 52.2 ± 5.8 ppb reported in our study they found intermittent inspiratory NO peaks of 160 ± 20 ppb [11]. This discrepancy is explained by the different sampling flow rates of 190 and 20 ml min^{-1}, respectively. Due to the response characteristics of the equipment used in this study (rise time for NO measurement T_{10-90} = 0.2 s) NO peaks found during nasal inspiration are but a lower estimate of the real inhaled NO concentrations. Higher NO concentrations may theoretically be present in inhaled gas for very short periods of time thus evading detection with the current equipment. Autoinhaled NO concentrations of 12 ± 1.7 ppb during regular breathing and intermittent maxi-

ma of 52.2 ± 5.8 ppb are of the same order of magnitude as concentrations of exogenous NO influencing oxygenation and pulmonary artery pressure in adult ARDS patients [22]. Moreover, improvements of oxygenation have been seen in long-term intubated patients receiving NO-containing nasal gas at a final concentration of 19 ppb [8]. Hence the inhalational NO concentrations found in neonates in this study may have biological effects in the lower respiratory system. The basis of the sudden inhalational NO peaks seen occasionally (fig. 1C, D) may be variable: regurgitation of gastric NO [23] may explain some peaks (fig. 1C). Moreover, breathholding (fig. 1D) may lead to accumulation of NO produced in the upper airways which will be autoinhaled with the consecutive breath. Accumulation of nasal NO has been shown after transient nasal occlusion in term and preterm infants during the neonatal period [11, 12].

In adult human subjects upper airway NO is mainly produced in the paranasal sinuses [5, 6]. In newborns the maxillary and ethmoid sinuses are of considerable size [24] and their patency is attested by the occasional occurrence of rhinosinusitis in this age group [25, 26]. It is thus likely that nasal NO production also occurs in the paranasal sinuses in the newborn. The sudden shifts of autoinhaled nasal NO peaks observed in this study may suggest that NO release from its production site was temporarily impeded. The transitions from high to low inspiratory nasal NO peaks or vice versa are reminiscent of the so-called nasal cycle, i.e. the cyclic reciprocal changes of nasal gas flow between the sides of the nose [27]. These are known to occur in both adults, children and infants [28–30]. Cyclic mucosal alterations have been shown to involve turbinates, nasal septum, lateral nasal wall (where the openings of the maxillary sinus and the anterior ethmoid cells are located), nasal cavity floor and also the ethmoid sinuses in adults [31] and might affect NO release from the paranasal sinuses. Alternatively, if the upper airway NO is constantly released from both sides, transient obstruction of the openings on one side, e.g. by mucus or the catheter, might have led to temporary shortage of autoinhaled nasal NO. This could be clinically relevant because a majority of premature infants are fed using similar tubes due to maturity-related feeding difficulties, thus intermittently depriving them of nasal NO.

In summary, this study shows that tidal NO analysis can noninvasively differentiate between NO derived from upper and lower airway sources in term and preterm neonates. Autoinhaled NO concentrations were similar to those shown to have biological effects in the respiratory system in adults. The measurement of lower airway NO was reproducible and may prove useful in future longitudinal studies on the relation between exhaled NO and inflammatory processes in neonatal lung disease.

Acknowledgments

We thank Lars-Erik Åkerlund and Armin Guhl for technical assistance. Andreas Artlich's tenure was funded by the Deutsche Forschungsgemeinschaft grant AR 205/2-1. This study was supported by the Karolinska Institute, Stiftelsen Vårdal, Sällskapet Barnavård and the Swedish MRC (7919).

References

1 Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S: Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem Biophys Res Commun 1991;181:852–857.

2 Gustafsson LE: Exhaled nitric oxide production by the lung; in Zapol WM, Bloch KD (eds): Nitric Oxide and the Lung. Basel, Dekker, 1997, pp 185–201.

3 Alving K, Weitzberg E, Lundberg JM: Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 1993;6:1368–1370.

4 Gerlach H, Rossaint R, Pappert D, Knorr M, Falke KJ: Autoinhalation of nitric oxide after endogenous synthesis in nasopharynx. Lancet 1994;343:518–519.

5 Lundberg JON, Rinder J, Weitzberg E, Lundberg JM, Alving K: Nasally exhaled nitric oxide in humans originates mainly in the paranasal sinuses. Acta Physiol Scand 1994;152:431–432.

6 Lundberg JON, Farkas-Szallasi T, Weitzberg E, Rinder J, Lidholm J, Änggård A, Hökfelt T, Lundberg JM, Alving K: High nitric oxide production in human paranasal sinuses. Nat Med 1995;1:370–373.

7 Schedin U, Frostell C, Persson MG, Jakobsson J, Andersson G, Gustafsson LE: Contribution from upper and lower airways to exhaled endogenous nitric oxide in humans. Acta Anaesthesiol Scand 1995;39:327–332.

8 Lundberg JON, Settergren G, Gelinder S, Lundberg JM, Alving K, Weitzberg E: Inhalation of nasally derived nitric oxide modulates pulmonary function in humans. Acta Physiol Scand 1996;158:343–347.

9 Settergren G, Angdin M, Astudillo R, Gelinder S, Liska J, Lundberg JON, Weitzberg E: Decreased pulmonary vascular resistance during nasal breathing: Modulation by endogenous nitric oxide from the paranasal sinuses. Acta Physiol Scand 1998;163:235–239.

10 Fineman JR, Soifer SJ, Heymann MA: Nitric oxide in the developing lung; in Zapol WM, Bloch KD (eds): Nitric Oxide and the Lung. Basel, Dekker, 1997, pp 87–95.

11 Schedin U, Norman M, Gustafsson LE, Herin P, Frostell C: Endogenous nitric oxide in the upper airways of healthy newborn infants. Pediatr Res 1996;40:148–151.

12 Schedin U, Norman M, Gustafsson LE, Jonsson B, Frostell C: Endogenous nitric oxide in the upper airways of premature and term infants. Acta Paediatr 1997;86:1229–1235.

13 Artlich A, Busch T, Lewandowski K, Schaible T, Falke KJ, Gortner L: Exhaled nitric oxide in preterm infants. Respir Physiol 1998;114:195–200.
14 Storme L, Riou Y, Leclerc F, Dubois A, Deschildre A, Pierre MH, Logier R, Robin H, Lequien P: Exhaled nitric oxide (NO) and respiratory function measured with body plethysmography in children. Arch Pediatr 1998;5:389–396.
15 Wildhaber JH, Hall GL, Stick SM: Measurements of exhaled nitric oxide with the single-breath technique and positive expiratory pressure in infants. Am J Respir Crit Care Med 1999;159:74–78.
16 Moa G, Nilsson K: Nasal continuous positive pressure: Experiences with a new technical approach. Acta Paediatr 1993;82:210–211.
17 Adding LC, Agvald P, Persson M, Gustafsson LE: Regulation of pulmonary nitric oxide by carbon dioxide is intrinsic to the lung. Acta Physiol Scand 1999;167:167–174.
18 Bland JM, Altman DG: Measurement error. BMJ 1996;313:744.
19 Trolin G, Anden T, Hedenstierna G: Nitric oxide in expired air at rest and during exercise. Acta Physiol Scand 1994;151:159–163.
20 Persson MG, Wiklund NP, Gustafsson LE: Endogenous nitric oxide in single exhalations and the change during exercise. Am Rev Respir Dis 1993;148:1210–1214.
21 Zetterquist W, Pedroletti C, Lundberg JON, Alving K: Salivary contribution to exhaled nitric oxide. Eur Respir J 1999;13:327–333.
22 Gerlach H, Rossaint R, Pappert D, Falke KJ: Time-course and dose-response of nitric oxide inhalation for systemic oxygenation and pulmonary hypertension in patients with adult respiratory distress syndrome. Eur J Clin Invest 1993;23:499–502.
23 Lundberg JON, Weitzberg E, Lundberg JM, Alving K: Intragastric nitric oxide production in humans: Measurements in expelled air. Gut 1994;35:1543–1546.
24 Parsons Schaeffer J: The Nose, Paranasal Sinuses, Nasolacrimal Passageways, and Olfactory Organ in Man. A Genetic, Developmental, and Anatomico-Physiological Consideration. Philadelphia, Blakiston, 1920.
25 Graney DO: Anatomy; in Cummings CW, Krause CJ (eds): Otolaryngology – Head and Neck Surgery, vol I: General, Face, Nose, Paranasal Sinuses. St Louis, Mosby, 1986, pp 845–850.
26 Kendall KA, Senders CW: Orbital and intracranial complications of sinusitis in children and adults; in Gershwin ME, Incaudo GA (eds): Diseases of the Sinuses. Totowa, Humana Press, 1996, pp 247–271.
27 Eccles R: A role for the nasal cycle in respiratory defence. Eur Respir J 1996;9:371–376.
28 Kayser R: Die exacte Messung der Luftdurchgängigkeit der Nase. Arch Laryngol Rhinol (Berl) 1895;3:101–120.
29 Stoksted P: Physiologic cycle of the nose under normal and pathologic conditions. Acta Otolaryngol (Stockh) 1952;42:175–179.
30 Fox GP, Matthews TG: The 'nasal cycle' in infants. Ir Med J 1991;84:24–25.
31 Zinreich SJ, Kennedy DW, Kumar AJ, Rosenbaum AE, Arrington JA, Johns ME: MR imaging of normal nasal cycle: Comparison with sinus pathology. J Comput Assist Tomogr 1988;12:1014–1019.